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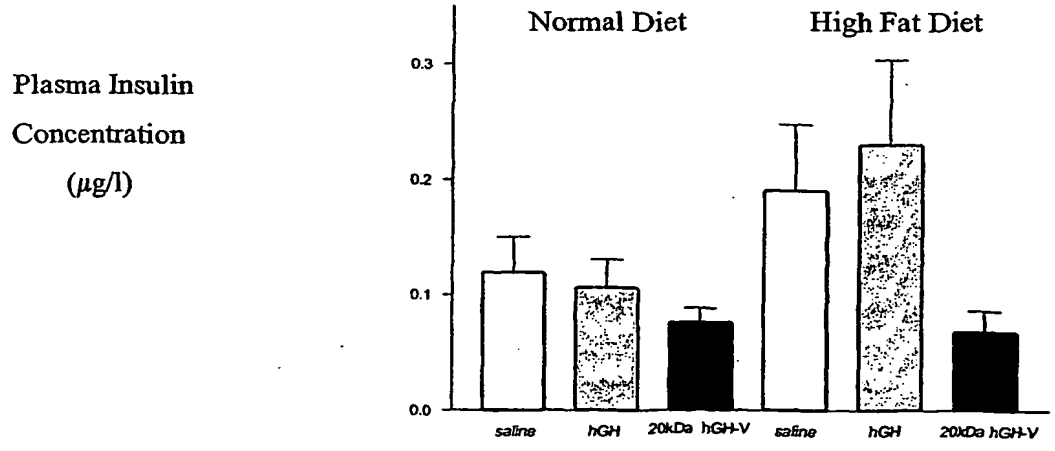
(54) Title: NON-DIABETOGENIC THERAPY USING A 20KDA PLACENTAL GROWTH HORMONE VARIANT



(57) Abstract: Embodiments of the present invention provide improved methods of treating conditions requiring human growth hormone (hGH) therapy, whereby the beneficial effects of hGH such as growth promotion and lipolysis are retained and unwanted properties such as insulin resistance are reduced or eliminated. In particular, embodiments of this invention are directed to methods of treatment whereby the diabetogenic side effects of conventional hGH treatment are reduced. The methods include the use of the growth hormone variant; 20kDa hGH-V in the treatment of conditions that are currently treated with hGH or that have the potential to be treated with hGH.

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NON-DIABETOGENIC THERAPY USING A 20KDA PLACENTAL GROWTH HORMONE VARIANT

CLAIM OF PRIORITY

This application claims priority to United States Provisional Patent Application Serial No: 60/590,794 filed July 23, 2004, Peter Gluckman and Stewart Gilmour, Inventors, titled "Enhanced Growth Hormone Therapy Using a 20kDa Placental Variant of Growth Hormone" (Attorney Docket No: ERNZ-01016US1); and

United States Provisional Patent Application Serial No: 60/649,469, filed February 2, 2005, Peter Gluckman, Stewart Gilmour and Mark Vickers, Inventors, titled "Growth Hormone Therapy Using an Antidiabetogenic 20kDa Placental Variant" (Attorney Docket No: ERNZ-01016US2). Both of these applications are incorporated herein fully by reference.

FIELD OF THE INVENTION

This invention pertains to conditions and diseases for which growth hormone is a desirable method of treatment. In particular, the invention pertains to the treatment of such conditions and diseases using variants of growth hormone. More particularly, the invention pertains to the treatment of such conditions and diseases using a 20 kilodalton placental growth hormone variant ("20kDa hGH-V") that does not display the diabetogenic effects of conventional growth hormone therapy.

BACKGROUND

There are several naturally occurring isoforms of growth hormone produced by two genes, one expressed in the pituitary, human growth hormone-N ("hGH-N" also known as "hGH-1"), and one expressed in the placenta, human growth hormone-V ("hGH-V" also known as "hGH-2"). The major form of hGH-N is a 22kDa protein consisting of 191 amino acids. A second form of hGH-N is produced by alternative splicing of the same gene, this results in deletion of a region corresponding to amino acids 32-46 of 22kDa hGH to produce a 20-kDa protein (20kDa hGH-N) (U.S. Pat. Nos. 6,399,565 and 6,436,674). Various other splice variants of hGH-N have been described (U.S. Pat. Nos. 4,670,393 and 5,962,411).

The hGH-V gene encodes for a 22kDa hGH-V isoform, which differs from 22kDa hGH-N by 13 amino acids in various positions throughout the hormone sequence (U.S. Pat No. 4,670,393). The 22kDa hGH-V is secreted by the placenta and appears in maternal serum at

mid-pregnancy. The exact function of this variant is still to be elucidated but it is believed to play a role in the control and development of fetal growth.

GH therapy is used in the treatment of a variety of conditions. However, conventional GH therapy is subject to the presence of detrimental side effects. It is known that individuals treated with conventional growth hormone can exhibit undesirable side effects of treatment, including a diabetogenic effect. It is clearly advantageous to establish a method of eliminating or at least alleviating these side effects. This unwanted side effect can lead to significant morbidity in subjects treated with growth hormone. Therefore, there is an urgent need in the art for growth hormone therapies that have reduced side effects, including a diabetogenic effect.

SUMMARY

GH therapy is used in the treatment of a variety of conditions. However, it is known that individuals treated with conventional growth hormone can exhibit undesirable side effects of treatment, including increase in insulin resistance, referred to as "diabetogenic effects" of GH therapy. It is clearly advantageous to establish a method of eliminating or at least alleviating these side effects, while maintaining in these patients desired somatogenic or metabolic effects of GH therapy.

Embodiments of this invention include methods of providing the benefits of growth hormone therapy while reducing the side effects of conventional GH treatment by the use of a variant of GH that has a different spectrum of activity to the 22kDa hGH-N, which is currently used for GH therapy. This variant provides the beneficial effects of conventional therapy such as growth promotion, stimulation of secretion of IGF-1 and lipolysis but unwanted properties, including diabetogenic effects are reduced. Hence, this invention is directed at the use of 20kDa hGH-V in the treatment of conditions that are currently treated with hGH or have the potential to be treated with hGH. In particular, it is directed at methods of treatment whereby the diabetogenic side effects of hGH treatment are reduced.

Embodiments of this invention also include methods of increasing levels of growth hormone in a mammal for prophylactic or therapeutic purposes, comprising administering to a mammal a pharmaceutically effective amount of a GH variant, 20kDa hGH-V, or a polypeptide that is substantially identical to a 20kDa hGH-V.

In one embodiment, this variant elicits the growth-promoting and lypolitic ability of GH but has a reduced ability to elicit undesired diabetogenic effects of GH in GH therapy.

In another embodiment, a 20kDa hGH-V is produced exogenously and administered to the subject. In view of the size of the variant, it is preferably produced by expression of a gene encoding the variant in a suitable host cell. Such a variant gene can be prepared by site-specific mutagenesis of a GH gene. Suitable host cells containing an expression vector containing such a variant gene can be implanted in the animal to be treated, and induction of expression of the variant gene can lead to increased levels of 20kDa hGH-V product, which can exert therapeutic effects.

BRIEF DESCRIPTION OF THE FIGURES

Aspects of this invention are described with reference to specific embodiments thereof. Other features of this invention can be appreciated with reference to the Figures in which:

Figure 1 depicts nucleotide sequences of hGH variants. Dashes indicate section deleted in 20kDa hGH-V and 20kDa hGH-N. Underlined section indicates signal sequences.

Figure 2 depicts predicted amino acid sequences for 22kDa hGH-V, 22kDa hGH-N, 20kDa hGH-V and 20kDa hGH-N. Dashed section indicates amino acids deleted in 20kDa hGH-V. Amino acid substitutions are indicated.

Figure 3a depicts a graph of body weight in animals fed a normal diet and treated with saline, 22kDa hGH-N or 20kDa hGH-V.

Figure 3b depicts a graph of body weight in animals fed a high fat diet and treated with saline, 22kDa hGH-N or 20kDa hGH-V.

Figure 4a depicts a graph of daily weight gain in animals fed a normal diet and treated with saline, 22kDa hGH-N or 20kDa hGH-V.

Figure 4b depicts a graph of daily weight gain in animals fed a high fat diet and treated with saline, 22kDa hGH-N or 20kDa hGH-V.

Figure 5 depicts a graph of retroperitoneal fat (as a % of total body weight) in animals treated with saline, 22kDa hGH-N or 20kDa hGH-V and either a normal diet or a high fat diet.

Figure 6 depicts a graph of blood hematocrit (%) in animals treated with saline, 22kDa hGH-N, or 20kDa hGH-V and either a normal diet or a high fat diet.

Figure 7 depicts a graph of fasting plasma insulin levels (in μg/l) in animals treated with saline, 22kDa hGH-N or 20kDa hGH-V and either a normal diet or a high fat diet.

Figure 8 depicts a graph of fasting plasma C-peptide (pg/ml) in animals treated with saline, 22kDa hGH-N or 20kDA hGH-V and either a normal diet or a high fat diet.

Figure 9 depicts a graph of fasting plasma triglycerides (mmol/l) in animals treated with saline, 22kDa hGH-N or 20kDa hGH-V and either a normal diet or a high fat diet.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

Definitions

"Transformation" means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integration. Methods of transformation may be the method of, for example, Graham and van der Eb, *Virology* 52: 456-457 (1973) or by other suitable methods for introducing DNA into cells such as by nuclear injection or by protoplast fusion. For cells which contain substantial cell wall constructions, such as prokaryote cells, transfection can be by calcium treatment as described by Cohen *et al*, *Proc. Natl. Acad. Sci.* (USA), 69: 2110 (1972). Additional methods are well known in the art and can be found in Sambrook and Russell, *Molecular Cloning Third Edition*, Cold Springs Harbor Laboratory Press, New York (2001).

"Transfection" means the introduction of DNA into a host cell whether or not any coding sequences are ultimately expressed. Cells do not naturally take up DNA thus, a variety of technical methods are utilised to facilitate gene transfer. Methods of transfection will be known to those skilled in the art and include for example, $CaPO_4$ and electroporation. Additional methods are well known in the art and can be found in Sambrook and Russell, *Id.*

"Conservative amino acid substitutions" mean amino acid substitutions or deletions that do not substantially affect the character of the variant polypeptide relative to the starting peptide. For example substitutions can be made within the following four groups: 1) positively charged residues e.g. Arg, Lys, His, 2) negatively charged residues e.g. Asn, Asp, Glu, Gln, 3) bulky aliphatic residues e.g. Ile, Leu, Val and 4) bulky aromatic residues e.g. Phe, Tyr, Trp. For further identification of conservative substitutions see, for example, Livingstone and Barton, *Comput. App. Biosci.* 9(6) 745-756, 1993.

"Substantially identical" refers to a polypeptide that has a sequence wherein one or two amino acid insertions, substitutions or deletions have been made or where conservative amino acid substitutions have been made such that the polypeptide thus formed does not materially differ in character and activity from 20kDa hGH-V and has at least 95% homology to the nucleotide sequence SEQ ID NO: 3. "Substantially identical" also refers to an oligonucleotide sequence that differs from a first oligonucleotide sequence by "silent" differences based on redundancy of the genetic code (e.g., in which the difference does not result in any change in amino acid). Alternatively, "substantially identical" sequences are oligonucleotide sequences that can hybridise to a complement of a first oligonucleotide sequence under stringent

conditions, for example, 0.1 SSC, 60°C for 1 hour. It can be appreciated that other conditions of stringency can be selected and still retain high-fidelity hybridization.

"hGH-N" means pituitary human growth hormone.

"hGH-V" means placental human growth hormone.

"20kDa hGH-V" means a polypeptide with the amino acid sequence of SEQ ID NO:7, or the nucleotide sequence of SEQ ID NO:3, or a polypeptide that is substantially identical to a polypeptide with the amino acid sequence of SEQ ID NO:7, or substantially identical to the nucleotide sequence of SEQ ID NO:3.

"20kDa hGH-N" means a polypeptide with the amino acid sequence of SEQ ID NO:8, or the nucleotide sequence of SEQ ID NO:4, or a polypeptide that is substantially identical to a polypeptide with the amino acid sequence of SEQ ID NO:8, or substantially identical to the nucleotide sequence of SEQ ID NO:4.

"22kDa hGH-V" means a polypeptide with the amino acid sequence of SEQ ID NO:5, the nucleotide sequence of SEQ ID NO:1, or a polypeptide that is substantially identical to a polypeptide with the amino acid sequence of SEQ ID NO:5, or substantially identical to the nucleotide sequence of SEQ ID NO:1.

"22kDa hGH-N" means a polypeptide with the amino acid sequence of SEQ ID NO:6, or the nucleotide sequence of SEQ ID NO:2, or a polypeptide that is substantially identical to a polypeptide with the amino acid sequence of SEQ ID NO:6, or substantially identical to the nucleotide sequence of SEQ ID NO:2.

"Somatogenic effects" includes growth-promoting, body-weight increasing and osteo-anabolic actions.

"Lactogenic effects" includes effects of exogenous growth hormone that are associated with PRLR signalling. Those effects include but not limited to: mammary gland development, changes in osmotic balance and cell proliferation.

"Metabolic effects" include, but are not limited to stimulation of lipolysis, stimulation of secretion of IGF-1, and diabetogenic effects.

Growth Hormone Therapy

There is an unmet need for methods and medicaments that have the beneficial growth promoting effects of GH but have reduced side effects.

Therefore, in certain aspects, this invention provides a method of treating a condition in a mammal, comprising administering to the mammal a pharmaceutically effective amount of 20kDa hGH-V or a polypeptide that is substantially identical to 20kDa hGH-V.

Administration of the 20kDa hGH-V variant maintains the somatogenic effects of 22kDa hGH-N but does not display the diabetogenic properties. Thus, use of the 20kDa hGH-V can be highly desirable in situations in which undesirable side effects of conventional GH therapy would be harmful. The use of 20kDa hGH-V is particularly desirable in:

- Patients who suffer from pediatric or adult GH deficiency or other GH treated conditions and who are at risk of or suffer from diabetes mellitus, obesity, metabolic syndrome, other insulin resistance related conditions (e.g. polycystic ovary syndrome) or hypertension;
- Patients who suffer from diabetes mellitus, obesity including morbid obesity, Prader-Willi syndrome, metabolic syndrome, other insulin resistance related conditions (e.g. polycystic ovary syndrome), hypertension, lipodystrophy; dyslipidemia, Turner's syndrome; and
- Patients who suffer from myocardial insufficiency, myocardial infarct and cardiac failure.

In other aspects, the invention comprises a pharmaceutical composition comprising a 20kDa hGH-V and a pharmaceutically acceptable excipient.

In still further aspects, this invention includes pharmaceutical composition comprising a 20kDa hGH-V, a pharmaceutically acceptable excipient and a binder.

In further aspects, this invention includes a pharmaceutical composition comprising a 20kDa hGH-V, a pharmaceutically acceptable excipient and a capsule.

Further aspects of this invention include a method for treating a patient in need of growth hormone therapy, comprising administering to a patient a 20kDa hGH-V.

In certain of these aspects, a method includes administering an expression vector capable of producing 20kDa hGH-V.

In further aspects, the expression vector is in a host cell.

In yet other aspects, the expression vector is in a cell of the patient.

In other aspects, the invention includes administering to a mammal in need of growth hormone therapy, a composition comprising 20kDa hGH-V.

In still other aspects, the invention includes administering to said mammal, a cell having a replicable vector therein capable of producing 20kDa hGH-V.

Side Effects of GH

Undesirable side effects of conventional GH therapy using 22kDa hGH-N include one or more of: oedema, fluid retention, hypertension, benign intracranial hypertension; glucose

intolerance and/or diabetes; gynaecomastia; oedema, benign intracranial hypertension, musculoskeletal effects such as arthralgia, paresthesias and carpal tunnel syndrome or, myalgia paresthesias and carpal tunnel syndrome.

Oedema is defined as an accumulation of an excessive amount of watery fluid in cells, tissues or serous cavities (such as the abdomen). Symptoms include puffiness of the face around the eyes, or in the feet, ankles and legs. GH induced salt and water retention can cause benign intracranial hypertension. Benign intracranial hypertension is characterized by increased cerebrospinal fluid pressure in the absence of a space occupying lesion. It can present with headache, visual loss, nausea, vomiting and papilloedema.

There is increasing concern over the diabetogenic effects of GH therapy especially during childhood. GH therapy has been shown to cause glucose intolerance and reduce insulin sensitivity. An increased incidence has been established between GH therapy and type-2 diabetes mellitus in some groups of children and in adolescents (Cutfield 2000). Hyperglycaemia has also been observed in adults undergoing GH treatment.

Arthralgia is pain in one or more joints.

Myalgia is pain or discomfort moving any muscle(s).

Paresthesia is a term that refers to an abnormal burning or prickling sensation which is generally felt in the hands, arms, legs, or feet, but can occur in any part of the body. Carpal tunnel syndrome occurs when tendons or ligaments in the wrist become enlarged, often from inflammation. The narrowed tunnel of bones and ligaments in the wrist pinches the nerves that reach the fingers and the muscles at the base of the thumb. Symptoms range from a burning, tingling numbness in the fingers, especially the thumb and the index and middle fingers, to difficulty gripping or making a fist, to dropping things.

There has been some concern about the possibility of "cancer growth promotion" with growth hormone therapy, based upon a few cases of leukaemia reported in children treated with growth hormone therapy.

Conditions Treated using GH

GH therapy is used to treat a varied range of conditions. At present, the prophylactic or therapeutic efficacy of GH has been established or indicated with regard to conditions that include, but are not limited to: adult-onset growth hormone deficiency (caused mainly by pituitary adenoma, surgery, or radiation therapy); childhood-onset growth hormone deficiency caused by: (a) congenital conditions (anatomical abnormalities or genetic factors), (b) acquired conditions (CNS tumours, cranial irradiation, infiltrative diseases, trauma, hypoxic insult) or

(c) idiopathic causes; cystic fibrosis, osteoporosis, chronic kidney failure, depression, memory loss, catabolic states, anorexia, hypertension.

GH therapy is approved for use in growth hormone deficiency in children, Prader-Willi syndrome, growth hormone deficiency in adults, Turner syndrome, chronic renal insufficiency and AIDS-associated wasting. Growth hormone is also useful in the treatment of several other conditions. These conditions include constitutional delay of growth, cystic fibrosis, osteoporosis, depression, memory loss, catabolic states and hypertension.

GH Deficiency

Diagnosis of growth hormone deficiency requires growth hormone stimulation testing. Tests used include the insulin hypoglycaemia test or insulin tolerance test (ITT), L-dopa stimulation test, arginine infusion test and arginine/GHRH test. Peak growth hormone secretion levels in adults of less than 3-5 ng/mL are indicative of GHD. In children values below 10 ng/ml are considered inadequate. Growth hormone deficiency is treated with recombinant human growth hormone, which is usually given *via* a subcutaneous injection on a daily basis.

There are several causes of GHD in children and most can be related to a problem in the hypothalamus or the pituitary. In certain rare cases, a defect in the body's utilization of growth hormone occurs. In most children with growth hormone deficiency, the defect lies in the hypothalamus. When other pituitary hormones are also not being secreted normally, the child is said to have hypopituitarism. In congenital hypopituitarism, abnormal formation of the pituitary or hypothalamus occurs during fetal development. Acquired hypopituitarism results from damage to the pituitary or hypothalamus that occurs during or following birth. It can be caused by a severe head injury, brain damage due to disease, radiation therapy, or a tumour.

The worldwide incidence of GHD in children has been estimated to be at least 1 in 10,000 live births and some individual countries have reported an incidence as high as 1 in 4,000 live births. A growth hormone deficient child usually shows a growth pattern of less than 2 inches a year. In many cases the child will grow normally until the age of 2 or 3 and then begin to show signs of delayed growth. Testing for growth hormone deficiency will occur when other possibilities of short stature have been ruled out. A weekly dose of up to 0.30 mg/kg of body weight divided into daily subcutaneous injections is recommended for GHD children.

In adults, deficiency of growth hormone can develop in the following situations; presence of a large pituitary tumour, after surgery or radiation therapy of pituitary tumour or other brain tumours, secondary to hypothalamic disorders and the continuation of childhood growth hormone deficiency into adulthood. The clinical features of adult GHD include; fatigue, muscle weakness, reduced exercise capacity, weight gain, increase in body fat and decrease in muscle mass, increase in LDL cholesterol and triglycerides and decrease in HDL cholesterol, increased risk for heart attack, heart failure and stroke, decrease in bone mass, anxiety and depression, especially lack of sense of well-being, social isolation and reduced energy. In the United States, an estimated total of 35,000 adults have GHD and approximately 6,000 new cases of GHD occur each year. For the average 70 kg man, the recommended dosage at the start of therapy is approximately 0.3 mg given as a daily subcutaneous injection. The dose can be increased, on the basis of individual requirements, to a maximum of 1.75 mg daily in patients younger than 35 years of age and to a maximum of 0.875 mg daily in patients older than 35 years. Lower doses may be needed to minimize the occurrence of adverse events, especially in older or overweight patients.

Prader-Willi Syndrome

Prader-Willi syndrome is a disorder of chromosome 15 characterised by hypotonia, hypogonadism, hyperphagia, cognitive impairment and difficult behaviour; the major medical concern being morbid obesity. Growth hormone is typically deficient, causing short stature, lack of pubertal growth spurt, and a high body fat ratio, even in those with normal weight. The need for GH therapy should be assessed in both children and adults. In children, if growth rate falls or height is below the third percentile, GH treatment should be considered. Growth hormone replacement helps to normalize the height and increases lean body mass; these both help with weight management. The usual weekly dose is 0.24 mg/kg of body weight; this is divided into 6 or 7 smaller doses over the course of the week.

Turner Syndrome

Turner syndrome occurs in approximately 1 in 2,500 live-born girls. It is due to abnormalities or absence of an X chromosome and is frequently associated with short stature, which can be ameliorated by GH treatment. Other features of Turner syndrome can include shortness of the neck and at times, webbing of the neck, cubitus valgus, shortness of fourth and fifth metacarpals and metatarsals, a shield shaped chest and primary hypogonadism. Growth in height is variable in patients with Turner syndrome so the decision whether to treat with GH

and the timing of such treatment is made on an individual basis. Often, treatment is initiated when a patient's height declines below the 5th percentile or when the standard deviation score decreases to less than 2 standard deviations below the mean. Treatment is often initiated with GH doses slightly higher than those used in treating GHD; a common starting dosage is 0.375 mg/kg per week divided into daily doses.

Chronic Renal Insufficiency

Chronic renal insufficiency (CRI) affects about 3,000 children in the United States. It manifests through a gradual and progressive loss of the ability of the kidneys to excrete wastes, concentrate urine, and conserve electrolytes. Approximately a third of children with chronic renal disease have abnormal growth partly because renal diseases disturb the metabolism of growth hormone. The corticosteroid hormones, which are often used to treat the kidney disease, can also retard growth. Kidney transplants can help a child start growing normally again, but most children do not make up the growth lost prior to transplantation. The age that the renal disease starts has more impact on growth retardation than the reduction in renal function (i.e. the younger the child when the disease starts, the more retarded is his or her growth). GH treatment can be given at a dosage of 0.35 mg/kg per week given six or seven times weekly.

HIV Wasting

A common problem among HIV-infected people is the HIV wasting syndrome, defined as unintended and progressive weight loss often accompanied by weakness, fever, nutritional deficiencies and diarrhoea. The syndrome, also known as cachexia, can diminish the quality of life, exacerbate illness and increase the risk of death for people with HIV. The body consumes muscle and organ tissue for energy instead of primarily relying on the body's stored fat.

Wasting can occur as a result of HIV infection itself but also is commonly associated with HIV-related opportunistic infections and cancers. HIV wasting syndrome is diagnosed in HIV-infected people who have unintentionally lost more than 10 percent of their body weight. Most patients with advanced HIV disease and AIDS eventually experience some degree of wasting. Estimates of the prevalence of AIDS wasting range from 4-30% of HIV infected individuals. GH treatment is in the order of 0.1 mg/kg daily.

Constitutional Delay of Growth

Constitutional delay of growth is characterized by normal prenatal growth followed by growth deceleration during infancy and childhood, and is reflected in declining height percentiles at this time. Between 3 years of age and late childhood, growth proceeds at a normal velocity. A period of pronounced growth deceleration can be observed immediately preceding the onset of puberty. Children with constitutional delay have later timing of puberty. At times, the combination of short stature accompanied and exaggerated by constitutional delay of growth and development in adolescents can cause sufficient psychosocial adolescent stress to warrant treatment with GH administered in the same manner and dosage as that used for treating GHD.

Cystic Fibrosis

Cystic Fibrosis (CF) is the most common lethal genetic disorder in America. An estimated 1000 individuals are born with Cystic Fibrosis each year in the United States. Cystic fibrosis causes dysfunction of the exocrine glands with increased viscosity of mucus secretions, which leads to pulmonary disease, exocrine pancreatic insufficiency, and intestinal obstruction. Early diagnosis and treatment has significantly decreased mortality in children with CF. However, malnutrition and poor growth continue to be a significant problem. Poor weight gain, weight loss, and inadequate nutrition result from reduced energy intake, increased energy loss, and increased energy expenditure. It has been reported that 28% of persons with CF are below the 10th percentile for height and 34% are below the 10th percentile for weight. Studies have shown that GH therapy improves height velocity, weight velocity, lean body mass (LBM) and pulmonary function in patients with cystic fibrosis.

Osteoporosis

Osteoporosis is a disease characterized by low bone mass and structural deterioration of bone tissue, leading to bone fragility and an increased susceptibility to fractures, especially of the hip, spine and wrist. Osteoporosis is responsible for more than 1.5 million hip fractures annually world wide. Most fractures occur in postmenopausal women, however, approximately one third of all osteoporotic fractures occur in men. Treatment of osteoporosis with GH might be beneficial due to the increased bone metabolism and improved bone geometry, which occurs with GH. The GH/ IGF-I system is dysregulated in patients with post-menopausal osteoporosis. This is shown by reduced systemic IGF and IGFBP-3-levels in osteoporosis suggesting a decrease of endogenous GH-secretion or a dysregulation of the GH

receptor system, which is beyond the normal ageing process of the GH/IGF system, the "somatopause". Studies have shown that GH treatments can improve bone mineral density in men with idiopathic osteoporosis.

Skeletal Dysplasias

Skeletal dysplasias associated with short stature such as achondroplasia can be treated with GH. Achondroplasia is a genetic disorder, affecting the fibroblast growth factor receptor type-III gene, which is evident at birth. It affects about one in every 20,000 births and it occurs in all races and in both sexes. During fetal development and childhood, cartilage normally develops into bone, except in a few places, such as the nose and the ears. In individuals with achondroplasia the rate at which cartilage cells in the growth plates of the long bones turn into bone is slow, leading to short bones and reduced height.

Achondroplasia is characterized by short stature, short limbs, proximal extremity (upper arm and thigh), head appears disproportionately large for body, skeletal (limb) abnormalities, abnormal hand appearance (trident hand) with persistent space between the long and ring fingers, marked kyphosis and lordosis (spine curvatures), waddling gait, bowed legs, prominent (conspicuous) forehead (frontal bossing), hypotonia and polyhydramnios (present when affected infant is born). GH has been approved to treat achondroplasia in some countries such as Japan and South Africa but does not yet have FDA approval.

Catabolic States

Catabolic states are characterised by protein wasting. Growth hormone treatment can be used to prevent excessive protein loss. Such catabolic states can exist in patients after long-term fasting, anorexia, chronic disease, prolonged immobilisation, trauma, burns and extensive surgery. GH and insulin-like growth factor I (IGF-I) play a physiological role in the regulation of protein metabolism in catabolic conditions. During such conditions the GH axis is frequently disturbed.

Lipodystrophy

GH can also be beneficial for the treatment of lipodystrophy, particularly for AIDS associated lipodystrophy. Lipodystrophy is a generic term that simply means a disturbance of fat metabolism. HIV-related lipodystrophy generally consists of fat accumulation in the following areas: subcutaneous tissues of the lower trunk (abdominal region), abdominal viscera (visceral obesity), axillary pads (bilateral, symmetric lipomatosis) and dorsocervical

region (-so-called buffalo hump) and loss of fat from the subcutaneous tissues of the following areas: lower extremities, upper extremities, buttocks and face (maxillary, nasolabial, and temporal regions). This syndrome of HIV-related lipodystrophy appears to be quite distinct from the wasting syndrome of protein-energy malnutrition. There is no universally agreed-on case definition of lipodystrophy in the HIV-infected patient so the diagnosis depends, to a certain extent, on a physician's clinical judgment. Skin-fold measurements or hip-to-waist ratios are neither very accurate nor reproducible. Single-slice CT scan at the level of the fourth lumbar vertebra is the most reproducible test, but it is also expensive.

Intrauterine Growth Retardation (IUGR) and Children of Small Gestational Age (SGA Children)

GH treatment can be beneficial in children with inter uterine growth retardation or infants who are small for gestational age (a condition also termed Russell-Silver syndrome). One definition of inter uterine growth retardation is a weight below the 10^{th} percentile for gestational age or a birth weight 2 standard deviations below the mean for gestational age. Studies have shown that those children who don't show catch-up growth can benefit from GH treatment.

Osteogenesis Imperfecta

Osteogensis imperfecta (OI) is caused by mutations in the gene for type I collagen. It is associated with bone de-mineralization and, in many instances, with retarded bone growth. OI is characterized by bones that break easily often from little or no apparent cause. While the number of people affected with OI in the United States is unknown, the best estimate suggests a minimum of 20,000 and possibly as many as 50,000. It is often, though not always, possible to diagnose OI based solely on clinical features. Clinical geneticists can also perform biochemical (collagen) or molecular (DNA) tests that can help confirm a diagnosis of OI in some situations. In some cases osteogenesis imperfecta can be effectively treated with GH. In particular, patients can experience improved bone mineralization and improved growth.

Inflammatory Bowel Disease

GH can be used for the treatment of inflammatory bowel disease, Crohn's disease and short bowel syndrome. Inflammatory bowel disease (IBD) is a group of disorders that cause inflammation or ulceration of the digestive tract. Depending on the type of IBD, any part of the digestive tract from the mouth to the anus can be affected. The small and large intestines,

the rectum, and the anus are affected most often. Ulcerative colitis and Crohn's disease are the most common types of inflammatory bowel disease. The cause of IBD is not known, however, it is believed to develop in people who have a genetic tendency. In these individuals, the immune system can overreact to normal intestinal bacteria, causing inflammation. The main symptoms are abdominal pain, rectal bleeding, and diarrhoea or constipation. Fever and loss of appetite also can occur. Short bowel syndrome is characterized by massive loss of intestine, with impaired net absorptive capacity of the remaining gut. Patients without colon often face problems with sodium/fluid balance and often require nutritional support due to malnutrition of several nutrients.

Glucocorticord Induced Growth Retardation

GH treatment can be considered in extremely short persons with growth retardation attributable to glucocorticoid treatment. The glucocorticoid regimen should be reduced to the minimal dose needed to achieve a satisfactory clinical effect before initiation of GH therapy in such patients.

Metabolic Conditions

Use of non-diabetogenic 20kDa hGH-V in patients suffering from metabolic conditions such as diabetes mellitus, insulin resistance and insulin resistance related conditions, dyslipideamia, obesity or metabolic syndrome give the patients benefit of the strong lipolytic effects of GH without subjecting them to the diabetogenic side effects of the standard GH therapy.

Diabetes Mellitus

Type II diabetes is diagnosed through physical examination combined with the fasting glucose test, or glucose tolerance test. Normal fasting glucose levels in children older than 2 years and adults range from 70mg/100ml-105mg/100ml. Levels between 105 mg/100ml and 126 mg/100ml are considered to have impaired fasting glucose and are at risk for later developing diabetes. Excess of 126 mg/100ml in fasting glucose levels on two different days is indicative of developed diabetes.

Glucose tolerance test measures patient's ability to tolerate a standard oral glucose load by obtaining serum and urine specimens for glucose level determinations before glucose administration and 5 subsequent time intervals. Peak and 2 hour value greater than 200mg/100ml on more than one occasion is indicative of diabetes mellitus.

The main risk factors for Type 2 diabetes are massive obesity; family history of diabetes; certain ethnicities, e.g. Hispanic, Polynesian; older age; people with high blood pressure or high cholesterol levels; women who have babies heavier than 4,000 grams.

Obesity

The most common statistical estimate of obesity is the body mass index (BMI), calculated by dividing the weight by the height squared; its unit is therefore kg/m^2. It is generally accepted that a BMI over 25.0 kg/m^2 indicates that a person is overweight, a BMI over 30.0 kg/m^2 denotes obesity. BMI of 40.0 kg/m^2 is identified as urgent morbidity risk ("morbid obesity").

Conditions such as hypothyroidism, Cushing's syndrome, the Prader-Willi syndrome and certain medications are known to cause weight gain which may lead to obesity. Genetic, psychological and environmental factors also play an important role in predisposing an individual to obesity.

Dyslipidemia

Dyslipidemia is characterised by increased LDL and triglyceride concentrations accompanied by decreased HDL. Standard tests can be carried out to measure levels of triglicerides and cholesterol in blood. Normal findings for male patients are: HDL higher than 45mg/100ml; LDL 60-180mg/100ml; VLDL 25%-50%; triglycerides – 40-160 mg/100ml.

Metabolic Syndrome

Metabolic syndrome, which is a combination of diabetes mellitus, hypertension, dyslipidemia and central obesity in patients, can be diagnosed by diagnostic methods specific for each of the coexisting symptoms.

Insulin resistance and insulin resistance-related conditions (e.g. polycistic ovary syndrome)

Insulin resistance can be tested using standard glucose tolerance test and fasting insulin test. The risk factors for development of insulin resistance include: obesity (body mass index of 30 kg/m^2) or more, a strong family history of diabetes, a history of gestational diabetes in pregnancy, hypertension, dyslipidemia, polycystic ovary syndrome, impaired glucose metabolism: fasting glucose level between 110 and 125 mg /100ml or impaired glucose tolerance, with a two-hour post-75-g glucose load level between 140 and 199 mg /100ml.

Hypertension

Hypertension is usually diagnosed on finding blood pressure above 140/90mmHg measured on both arms on three occasions over a few weeks. Pressures around 140-150/90-100mmHg would be called mild hypertension. Pressures around 150-170/100-110 mmHg would be called moderate, pressures higher than 200/120mmHg are considered severe.

The risk factors contributing to development of hypertension include: family history, environmental conditions (diet, obesity, lifestyle) pre-existing conditions (secondary hypertension (polycystic kidneys, renal artery stenosis (narrowing of the kidney artery), hyperthyroidism, hyperaldosteronism, Cushing's syndrome, sleep apnea). Therapeutic use of 22kDa hGH-N to reduce systolic blood pressure was disclosed in the prior art in Vickers et al. WO00/030588.

Other Conditions

Other conditions that can benefit from GH treatment include depression, memory loss, infertility and the like. However, it can be readily appreciated that any condition that can benefit from GH therapy can be treated advantageously using methods and medicaments of this invention.

Therapy Using Pituitary GH

Current conventional GH therapy uses 22kDa pituitary GH. The 22kDa and 20kDa versions of pituitary GH are thought to have equivalent somatogenic activity. 20kDa hGH-N was equivalent to 22kDa hGH-N in a growth-promoting assay in spontaneous dwarf rats (Ishikawa 2000, Ishikawa 2001), the osteo-anabolic effect of 20kDa was equipotent to that of 22 kDa (Wang 1999), cell proliferation of full length hGH-R-expressing cells was stimulated equipotently (Wada 1998) and 20kDa hGH-N was shown to be a full agonist in hypophysectomized rats (Uchida 1997). 20kDa hGH-N has also been shown to produce inhibition of LPL activity in adipose tissue and to stimulate lipolysis in adipocytes in a manner similar to 22kDa hGH-N (Takahashi 2002). The lipolytic activity of 20kDa hGH-N may be higher than 22kDa hGH-N in the presence of GHBP (Asada 2000).

However, 22kDa hGH-N is known to induce insulin resistance. Studies indicate that the diabetogenicity of 20kDa hGH-N is much weaker than 22kDa hGH-N. 20kDa hGH-N was shown to much less potent than 22kDa hGH-N at inducing insulin resistance in euglycaemic clamp studies (Takahashi 2001) and in studies using GH deficient dwarf rats (Ishikawa 2001).

20kDa hGH-N is a much weaker agonist for the prolactin receptor than 22kDa hGH-N and hence lacks some of the lactogenic properties of 22kDa hGH-N (Tsunekawa 1999) as the lactogenic effects of GH are believed to be mediated by the prolactin receptor. It has been suggested that administration of 20kDa hGH-N may alleviate hPRLR-mediated side-effects such as breast cancer (Tsunekawa 1999). 20kDa hGH-N also has different antidiuretic effects to 22kDa hGH-N. Administration of 22kDa hGH-N suppressed urine excretion in intact rats whereas 20kDa hGH-N showed no significant effect (Satozawa 2000), this is significant as fluid retention can cause oedema. 20kDa hGH-N is thought to lack part of the PRLR binding region of 22kDa hGH-N. 20kDa hGH-N is thought to lack part of the PRLR binding region of 22kDa hGH-N. Figures 1 and 2 below show the oligonucleotide and amino acid sequences of growth hormone variants useful in the methods of this invention. In Figure 1, sequence identification numbers below are for oligonucleotides: 22kDa hGH-V (SEQ ID NO:1), 22kDa hGH-N (SEQ ID NO:2), 20kDa hGH-V (SEQ ID NO:3) and 20kDa hGH-N (SEQ ID NO:4). Figure 2 shows amino acid sequences for 22kDa hGH-V (SEQ ID NO:5), 22kDa hGH-N (SEQ ID NO:6), 20kDa hGH-V (SEQ ID NO:7) and 20kDa hGH-N (SEQ ID NO:8).

Therapy Using 20kDa Placental Growth Hormone Variant

22kDa hGH-V has been shown to have similar somatogenic but reduced lactogenic activity compared to the hGH-N isoform (Igout 1995). 22kDa hGH-V binds to somatogen receptors (Ray 1990) and stimulated growth in hypophysectomized rats (MacLeod 1991). 22kDa hGH-V binds to both somatogen and lactogen receptors but the ratio of its somatogen to lactogen receptor-binding affinities is higher than that of 22kDa hGH-N. This ratio differed by 7-8 fold in experiments using rat liver lactogen receptors (Ray 1990) and by 30 fold using Nb2 cell lactogen receptors (MacLeod 1991). The lipolytic and insulin-like activities of 22kDa hGH-N and 22 kDa hGH-V have been shown to be similar in rat adipose tissue (Goodman 1991).

A second splice variant of the GH-V gene retains intron D in the mRNA to give a 26kDa hGH-V isoform (hGH-V2) (Cooke 1988). Recently two new transcripts of the hGH-V gene have been described (Boguszewski 1998). hGH-V3 is generated by alternative splicing near the end of the fourth exon to predict a 24kDa protein (219 amino acids) wherein the carboxy-terminal residues show complete sequence divergence from hGH-V. The second transcript to be described uses a similar alternative splice site within exon 3, to that seen for hGH-N, to predict a 20kDa isoform of hGH-V (GenBank accession number: AF006060).

The transcript for 20kDa hGH-V had not previously been detected and it was thought that the hGH-V gene did not use this splice site (Cooke 1998, Estes 1992) however, Boguszewski *et al* detected the transcript of this isoform in two of four full term placentas and in one abnormal placenta (Boguszewski 1998). The difference in expression of this transcript, as the transcript was not found in all placentas, may partly explain the lack of previous detection. While the transcript has been detected the encoded protein has not been isolated and hence, the biological activity was unknown.

It does not follow that the knowledge of the existence of the above transcript means that it can be synthesized or that the biological activity can be predicted. For example, 20kDa hGH-N proved difficult to obtain. 20kDa hGH-N can be purified from the pituitary in small amounts but complete separation from 22kDa hGH-N is difficult due to similarity in physiochemical properties between the two hormones. Methionyl 20kDa hGH-N has been expressed in *E. coli.* however, the additional methionine residue at the N-terminal may affect biological activity and it is believed that the protein may also be incorrectly folded as has been the case for methionyl 22kDa hGH-N (Hsiung 1988). Methionyl 20kDa hGH-N was expressed at only one-twentieth of 22kDa hGH-N and 20kDa hGH-N produced in COS-7 cells was reported to be secreted at one-thirtieth as compared to 22kDa hGH-N (Rincón-Limas 1993) hence, the development of an efficient synthesis by Uchida *et al* was not straightforward (Uchida 1997).

Early work on 20kDa hGH-N isolated from the pituitary and with a non-authentic recombinant product gave quite different results to studies of an 'authentic' version (Uchida 1997). Early studies on 20kDa hGH-N purified from the pituitary indicated that the lipolysis activity of 20kDa hGH-N was much weaker than 22kDa hGH-N (Frigeri 1979, Juárez-Aguilar 1995). This did not agree with results obtained using recombinant 20kDa hGH-N with an authentic sequence (Asada 2000, Takahashi 2002). Methionyl 20kDa hGH-N has been shown to induce glucose intolerance (Kostyo 1985) and impair insulin sensitivity (Ader 1987) however, more recent studies on 20kDa hGH-N indicate that the diabetogenicity of 20kDa hGH-N is much weaker than 22kDa hGH-N (Takahashi 2001, Ishikawa 2001). Such discrepancies in the literature describing the biological properties of 20kDa hGH-N produced by different methods indicate that it is obviously not straightforward to predict and demonstrate said properties.

Novel features of the present invention include the surprising finding that 20kDa hGH-V does not display the diabetogenic effects associated with GH-N therapy.

The use of the 20kDa hGH-V is desirable in:

- Patients who suffer from pediatric or adult GH deficiency or other GH treated conditions and who are at risk of or suffer from diabetes mellitus, obesity, metabolic syndrome, other insulin resistance related conditions (e.g. polycystic ovary syndrome) or hypertension;
- Patients who suffer from diabetes mellitus, obesity including morbid obesity, Prader-Willy syndrome, metabolic syndrome, other insulin resistance related conditions (e.g. polycystic ovary syndrome), hypertension, lipodystrophy; dyslipidemia, Turner's syndrome; and
- Patients who suffer from myocardial insufficiency, myocardial infarct and cardiac failure.

The above-identified disorders can be effectively treated in patients having diabetes or at risk of developing diabetes or diabetes-related conditions. The use of conventional GH therapy may be contraindicated in patients having diabetic or diabetic-related conditions described herein may be contraindicated. In some embodiments, treatment is with 20kDa hGH-V described in Figure 2 (SEQ ID NO:7), which depicts the predicted amino acid sequences for 22 kDa hGH-V, 22kDa hHG-N, 20kDa hGH-V and 20kDa hGH-N. A dash indicated deleted amino acids. In other embodiments, treatment is with an oligonucleotide selected from Figure 1, which depicts oligonucleotide sequences of hGH variants. Dashes indicate section deleted in the 20kDa hGH-V (SEQ ID NO:3) and 20kDa hGH-N (SEQ ID NO:4).

The Inventors have found that 20kDa hGH-V is more beneficial as GH replacement therapy than the conventional 22kDa hGH-N. The Inventor's discovery that 20kDa hGH-V has desirable somatogenic effects and less diabetogenic side effects shows that this variant can provide practitioners with desirable alternatives to conventional therapy.

Synthesis and Preparation of 20kDa hGH-V

Polypeptides of the present invention can be provided in an isolated form and in some embodiments can be purified. The term 'isolated' means that the material is removed from its original environment.

Polypeptides of the present invention can be derived from a naturally purified protein, a product of chemical synthesis or produced by recombinant techniques.

In one series of embodiments a polypeptide can be produced by recombinant techniques. Host cells are transformed with expression vectors and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants and/or amplifying the gene(s) that produce the 20kDa hGH-V. Culture conditions such as temperature, pH and the like, are those used for the host cell selected for expression and will be apparent to those skilled in the art. It can be appreciated that for purposes of this discussion, the term "DNA," "gene" and 'cDNA" may be equivalent to the term "RNA" or "mRNA" to the degree that the sequences of nucleotides in the oligonucleotides convey the information necessary to produce a polypeptide. Thus, if referring to RNA, the base uracil (U) is used, whereas if referring to DNA, the base thymine (T) is used. It can be appreciated that regardless of whether the oligonucleotide is RNA or DNA, the resulting polypeptide can be made using either type of oligonucleotide.

Examples of cloning and expression vectors for use with prokaryotic and eukaryotic hosts can be found in, for example, Sambrook *et al*, *Molecular Cloning: A Laboratory Manual,* Third Edition, Cold Springs Harbor, N. Y. (2001).

A polynucleotide (SEQ ID NO:3 or substantially identical polynucleotide) can be employed for producing a polypeptide by recombinant techniques. A polynucleotide can be included in any one of a variety of suitable vectors or plasmids for expressing a polypeptide. Such vectors include but are not limited to, chromosomal, non-chromosomal and synthetic DNA sequences e.g. derivatives of SV40, bacterial plasmids, phage DNAs, yeast plasmids, vectors derived from combinations of plasmids and phage DNAs, viral DNA such as vaccinia, adenovirus, fowl pox virus, pseudorabies and the like.

In certain embodiments, an oligonucleotide encoding 20kD hGH-V can be expressed to produce a mRNA that can be translated into a polypeptide containing the 26 amino acids of the signal sequence of 20kDa hHG-V (e.g., Met^{-26} – Ala^{-1}). Subsequent cleavage by an endopeptidase selective for internal Ala-Phe bonds can then be used to liberate the "mature" polypeptide for therapeutic use. One example of such an endopeptidase is neutral endopeptidase (E.C. 3.4.24.11), an enzyme that preferentially cleaves peptides between small aliphatic amino acids (e.g., Gly, Ala) and aromatic (Phe) or hydrophobic (e.g., Leu, Ile) amino acids. Other endopeptidases are known in the art and need not be described herein further.

In other embodiments, mature 20kDa hHG-V can be produced using an expression cassette comprising an initiation codon (ATG) followed by a codon for Phe (e.g., TTT or TTC). The remainder of the open reading frame is otherwise identical as that depicted in

Figure 1. Upon translation, the peptide can be cleaved using an aminopeptidase to remove the N-terminal Met residue, thereby producing the 'mature' 20kDa hGH-V.

In still further embodiments, an expression cassette can be constructed in which a 3' segment is added before the TTT or TTC codon for Phe^1, in which the segment encodes for a leader sequence that is normally cleaved by the cell expressing the polypeptide. Thus, the leader sequence is cleaved, producing the 'mature' polypeptide 20kDa hGH-V for subsequent use.

Useful expression vectors for bacterial use can be constructed by inserting a structural in frame DNA sequence encoding a desired protein together with suitable translation initiation and termination signals, for example start (ATG) and stop codons, operably linked to a functional promoter. If desired, enhancer elements can also be included to increase or otherwise regulate the expression of the oligonucleotide. A vector can comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host.

Suitable vectors will be known to those skilled in the art and many are available commercially. Suitable vectors include but are not limited to bacterial vectors: pBs, pQE-9 (Qiagen), phagescript, PsiX174, pBluescript SK, pBsKS, pNH8a, pNH16a, PNH18a, pNH46a (Stratagene), pTrc99A, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia); eukaryotic vectors: pWLneo, pSV2cat, pOG44, pXT1, pSG (Stratagene), pSVK3, pBPV, pMSG, pSVL (Pharmacia), pET20b(+) and the like.

An appropriate DNA sequence can be inserted into the vector by a variety of procedures. In general, a DNA sequence can be inserted into an appropriate restriction endonuclease site(s) by procedures that will be known to those skilled in the art. In one series of embodiments, restriction enzymes NcoI and HindIII can be used.

A DNA sequence in the expression vector can be operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. Examples of such promoters include LTR or SV40 promoter, the *E. Coli* lac, trp or RecA, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses.

The selection of the appropriate promoter will be within the scope of those skilled in the art. Examples of promoters include but are not limited to bacterial promoters such as lacI, lacZ, T3, T7, gpt, lambda P_R, trc and the like and eukaryotic promoters such as CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retroviruses, mouse metallothionein-I and the like.

However, the above are only recited as examples, and other promoters can be used. Methods for monitoring and quantifying expression of genes are known in the art and can be used to verify the levels of expression for producing 20kDa hGH-V.

An expression vector can also contain a ribosome-binding site for translation initiation and a transcription terminator. A vector may also include appropriate sequences for amplifying expression (enhancers).

Mammalian expression vectors can comprise an origin of replication, a suitable promoter and enhancer and any necessary ribosome binding site, polyadenylation site, splice donor and/or acceptor sites, transcriptional termination sequences and 5' flanking non-transcribed sequences.

In addition, an expression vector can contain a gene to provide a phenotypic trait for selection (selection marker) of transformed host cells. Suitable selection markers include dihydrofolate reductase (dfr) or neomycin resistance (neo) for eukaryotic cell culture or such as tetracycline or ampicillin resistance in *E. Coli.*

A vector can also include a leader sequence capable of directing secretion of translated protein into the periplasmic space, the cellular membrane or the extracellular medium.

A vector containing an appropriate DNA sequence as well as an appropriate promoter or control sequence, can be employed to transform an appropriate host to enable the host to express the protein. Suitable hosts include but are not limited to bacterial cells such as *E. Coli, Bacillus subtilis, Salmonella typhimurium,* various species within the genera Pseudomonas, Streptomyces, Staphlococcus *Salmonella typhimurium*; fungal cells such as yeast; animal cells such as COS-7 lines of monkey kidney fibroblasts and other cell lines capable of expressing a compatible vector such as the C127, 3T3, CHO, HeLa, BHK, BL21(DE3)pLysS competent cell lines; plant cells and the like. The selection of a suitable host will be within the scope of those skilled in the art. In one embodiment the host cell is *E. Coli.*

Introduction of a construct into a host cell can be effected by calcium phosphate transfection, DEAE, dextran mediated transfection or electroporation (Davis *et al, basic methods in Molecular Biology,* 1986). In one embodiment the construct is introduced using calcium.

A host cell may be induced to express a desired protein by various methods including but not limited to tryptophan starvation, isopropylthiogalactoside (IPTG), nalidixic acid and the like. In one series of embodiments, expression can be induced by nalidixic acid.

Transcription by eukaryotic cells of a DNA encoding a polypeptide of the invention can be increased by inserting an enhancer sequence into the vector. Suitable enhancers will be

known to those skilled in the art and include, but are not limited to, the SV40 enhancer on the late stage of the replication origin, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, adenovirus enhancers and the like.

It can be appreciated that workers of ordinary skill can use additional methods known in the art to produce expression systems and to use those systems to produce recombinant 20kDa hGH-V for therapeutic purposes.

It can also be appreciated that certain host cells can be implanted directly into the subject to be treated. For example, autologous cells can be harvested from a patient or heterologous cells can be transfected with an expression vector of this invention. Such cells can then be implanted into the patient and induction of production of 20kDa hGH-V can result in the production, *in vivo*, of therapeutic quantities of the 20kDa hGH-V.

Additionally, it is contemplated that gene therapy methods, using for example, a virus such as adenovirus, or a liposome can comprise an expression cassette for expression of 20 kDa hGH-V that can be transferred *in vivo* into a host cell of the animal to be treated.

Isolation of 20kDa hGH-V

Cells can be harvested by centrifugation, disrupted by physical or chemical means and a resulting crude extract can be purified. Microbial cells employed in expression of proteins can be disrupted by any convenient method including freeze-thaw cycling, sonication, mechanical disruption, use of cell lysing agents, (including T7 lysozyme expressed by BL21(DE3)pLysS when under chloramphenicol), detergents and the like.

A 20kDa hGH-V variant can be purified from recombinant cell cultures using a variety of methods, including but not limited to, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, affinity chromatography, hydrophobic interaction chromatography, phosphocellulose chromatography, hydroxyapatite chromatography, lectin chromatography, gel filtration and the like.

A recombinant protein produced in bacterial culture can be isolated by initial extraction from cell pellets, followed by one or more of salting-out, aqueous ion exchange or size exclusion chromatography steps. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. SDS-PAGE, ion exchange, chromatography or HPLC can be employed for final purification.

In other embodiments, a protein can be extracted from a bacterial culture by initially solubilizing the inclusion bodies followed by ion-exchange chromatography and gel filtration purification. The protein is then refolded using urea at high pH.

The sequence of the protein can be validated using any appropriate method including but not limited to N-terminal sequencing, proteolytic mapping and peptide sequencing. Functional characteristics can be evaluated using, for example, activation of GH receptors, immunological methods, stimulation of GH-sensitive cells in culture, and the like. In one series of embodiments, a protein can be validated by measuring the capacity of the protein to form a 1:2 complex with hGH binding protein. In other embodiments, a protein's function can be verified by its ability to activate cells transfected with GH receptors, for example, derived from a rabbit.

Depending on the host employed in a recombinant procedure to produce the polypeptide, the polypeptide of the invention may be glycosylated or non-glycosylated and may include an initial methionine amino acid (at position -1). It is known that certain prokaryotic host cells do not glycosylate proteins as well as do certain eukaryotic host cells. To promote higher degrees of glycosylation, one can provide greater levels of essential monosaccharides or their precursors into the growth medium. For example, for proteins that contain sialic acid, fucose, galactose or N-acetyl-galactosamine, a cell culture medium enriched in those nutrients can be desirably used to increase the level of expression of glycosylated forms of 20kDa hGH-V. It can be readily appreciated that other sugars needed to glycosolate a 20kDa hGH-V can be used to supplement the growth medium as well. Moreover, if desired, one can increase the expression of a host cell's glycosyltransferases and/or nucleoside triphosphate glycosylation enzymes (sugar loading enzymes) to increase the addition of sugar residues to a 20kDa hGH-V. Further descriptions of glycosylation can be found in Alberts et al., *Molecular Biology of the Cell*, Fourth Edition, Garland Science (2002).

There is some ambiguity as to the nature of the amino acid at position 14 of 20kDa hGH-N. Martial *et al* (Martial *et al, Science* 205, 602, 1979) reported that the mRNA sequence coding for the amino acid at this position was AUG coding for methionine and Masuda *et al* (Masuda *et al, Biophysica Acta,* 949, 125, 1988) reported that the cDNA sequence coding for the 14th amino acid from the N-terminal was AGT coding for serine. While it is believed that the amino acid in this position in the 20kDa hGH-V variant is a methionine, the invention is understood to include both variations.

Furthermore, amino acid sequences in which one or two amino acids are replaced, inserted or deleted should be understood to fall under the category of the variant 20kDa hGH-V. Conservative variants, silent mutations and conservative amino acid substitutions should also be understood to fall under the category of the variant of this invention.

Conservative variants of nucleotide sequences include nucleotide substitutions that do not result in changes in the amino acid sequence, as well as nucleotide substitutions that result in conservative amino acid substitutions, or amino acid substitutions which do not substantially affect the character of the polypeptide translated from said nucleotides.

Pharmaceutical Compositions and Administration

GH therapy can be divided into two categories: physiological and pharmacological. Physiological therapy replacement therapy involves lower dosages. Starting replacement therapy dosages for GH in children range from 0.02 to 0.05 mg/kg per day and in adults from 0.00625 to 0.025 mg/kg per day. For a 70 kg man, the usual starting dose is 0.3 mg/day with a maintenance dose of 0.35 to 0.56 mg/day. GH replacement can be given throughout the lifetime of some patients. Pharmacologic therapy, for example to treat AIDS associated wasting, involves higher dosages; in children >1 mg/day and in adults; >1 to 3 mg/day. At this higher dosage more and more pronounced side effects can be observed.

The invention also includes a 20kDa hGH-V variant described herein, where the variant is conjugated to one or more water soluble polymers in order to provide additional desirable properties of the variant while still maintaining agonist properties. Such properties include increased solubility, increased stability, reduced immunogenicity, increased resistance to proteolytic degradation, increased in vivo half-life and decreased renal clearance. Suitable polymers include, but are not limited to, polyethylene glycol, polypropylene glycol and polysaccharides. Methods of forming suitable conjugates will be known to those skilled in the art. Polyethylene glycol is particularly preferred and methods of conjugation are described in e.g. WO 95/32003.

In general, compounds of this invention can be administered as pharmaceutical compositions by one of the following routes: oral, topical, systemic (e.g. transdermal, intranasal or by suppository), parenteral (e.g. intramuscular, subcutaneous or intravenous injection), by implantation and by infusion through such devices as osmotic pumps, transdermal patches and the like. In certain embodiments, subcutaneous or intramuscular injection or injection using needle-free devices can be used, where a solution containing the compound is dispersed through the skin in a fine mist to enable subcutaneous delivery.

Compositions can take the form of tablets, pills, capsules, semisolids, powders, sustained release formulation, solutions, suspensions, elixirs, aerosols or any other appropriate compositions; and can include pharmaceutically acceptable excipients. In some embodiments, a composition is in powdered form to be reconstituted before administration or as a solution or

suspension containing the GH variant. Suitable excipients are well known to persons of ordinary skill in the art, and they, and the methods of formulating the compositions, can be found in such standard references as Gennaro AR: *Remington: The Science and Practice of Pharmacy,* 20th Ed., Lippincott, Williams and Wilkins, Philadephia, PA (2000). Preferred excipients include, but are not limited to, sodium chloride, phenol, *m*-cresol, benzyl alcohol, polysorbate 20, sodium citrate, mannitol, sodium dihydrogen phosphate, disodium hydrogen phosphate, glycine and glycerin. Suitable liquid carriers, especially for injectable solutions include sterile water, aqueous saline solution, aqueous dextrose solution and the like, with isotonic solutions being preferred for parenteral administration.

Compounds of this invention are also suitably administered by a sustained-release system. Suitable examples of sustained release compositions include semi-permeable polymer matrices in the form of shaped articles e.g. films or microcapsules. Sustained release matrices include polylactides (U.S. Pat. No. 3,773,919; EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate, poly(2-hydroxyethyl methacrylate), ethylene vinyl acetate or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include a liposomally entrapped compound. Liposomes containing the compound are prepared by methods known per se: DE 3,218,121; EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Apln. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545 and EP 102,324.

It can be appreciated that the above descriptions are for purposes of illustration only and are not intended to limit the scope of this invention. Rather, persons of ordinary skill can readily appreciate that modifications of the above methods and compositions can be readily used and prepared, and all such variations are considered within the scope of this invention. Further, all references cited herein are incorporated herein fully by reference.

EXAMPLES

Other aspects of this invention are described with respect to specific examples demonstrating properties of the methods and compositions of this invention. The examples that follow are intended to illustrate advantages of this invention and are not intended to limit the scope of the invention.

Example 1: Non-diabetogenic Effect of 20kDa hGH-V

The use of growth hormone therapy in the treatment of children with short stature and in adult growth hormone deficiency is widely established. However, despite the well-known effects of 22kDa hGH-N on improving body composition, lipid profiles, cardiovascular function and bone density there are several problems associated with 22kDa hGH-N therapy as it exists at present. These problems include:

(1) large pharmacological doses of 22kDa hGH-N may be associated with clinical sequelae of GH excess, including fluid retention.

(2) diabetogenic (anti-insulin) side effects of 22kDa hGH-N therapy including induction of peripheral insulin resistance and glucose intolerance.

The present example was designed to test the efficacy of the 20kDa hGH-V on linear growth and certain endocrine and metabolic markers in GH-normal Wistar rats fed either normal diet (chow) or a high fat diet.

Materials and Methods

Animal Model *The Wistar rat*

Male Wistar rats were purchased from a colony maintained by the Animal Resources Unit at the University of Auckland (ethics approval No. R38) at a weaning age (21-22) days. Animals were acquired at this age (i.e. 3-4 weeks prior to investigative age) to allow time for acclimatisation and familiarisation of handling with the investigating personnel. Animals were housed in a dedicated facility using standard rat cages, normal light-dark cycles and unlimited access to food and water. The animals were monitored daily from weaning until the completion of the studies. At weaning animals (n=18 per group) were weigh- matched to be fed with standard diet of rat chow (Harlan Teklad Diet 2018) or a commercial high fat diet (Research Diets D12451, 45% kcals as fat).

Treatment protocol

At 7-8 weeks of age, male Wistar rats were weight-matched within diet and assigned to one of 3 treatment groups (n=6 per group) to receive either vehicle (physiological saline (0.9%))) or GH. The treatment groups were as shown in Table 1.

Table 1. Treatment Groups.

Group	Dose
Saline	0

hGH (Genotropin)	5.0ug/g/day
20kDa hGH-V	5.0ug/g/day

hGH was reconstituted using physiological saline (0.9%). bGH was reconstituted using carbonate buffered saline (pH9.4). The placental 20kDa hGH variant was reconstituted in sterile water at a pH of 11.0. Injections (volume 100ul) were administered by subcutaneous injection given twice daily at 0800 and 1700h using a fine gauge diabetic syringe (29g). Animals were treated for 7 days and sacrificed on the morning of day 8 following a final GH injection.

Body Weight

Animals were weighed between 8-9am every day for the duration of the experiment. Individual animals were observed daily for any signs of clinical change, reaction to treatment or ill health. There were no indications whatsoever of any adverse stress responses and related symptoms in any of the treatment groups.

Food and Water Consumption

Food intake was measured on a daily basis for the duration of the trial. Relative food intake per rat (grams consumed per gram body weight per day) was calculated using the amount of food given to and the amount of food left uneaten by each pair in each group. Water consumption was calculated daily by weighing water bottles at the same time on each day of the study.

Body Length

Body lengths (nose-anus and nose-tail) were assessed post-mortem using standard measurement techniques.

Tissue and Plasma Measurements

On day 8, following an overnight fast, animals were sacrificed by halothane anaesthesia followed by decapitation. Measurements of body length, carcass weight, organ weights (liver, spleen, kidney, adrenals, heart, pituitary) and fat pad weight (retroperitoneal) were taken. Trunk blood was collected into heparinised vacutainers for endocrine and biochemistry analysis. Blood samples were analysed for insulin, glucose, FFAs, leptin, IGF-I, glycerol, triglycerides and total protein.

Results

Body weights were significantly increased in animals fed the high fat diet ($p<0.05$). Body weight was significantly increased in hGH treated animals ($p<0.05$) but was not significantly different from saline in the 20kDa GH treated animals.

Body Weight

Body weights of the animals studied are depicted in Figures 3a and 3b. Figure 3a shows that animals fed a normal diet and treated with hGH (filled squares) or 20kDa hGH-V (filled circles) grew faster than animals treated with saline (open circles). Figure 3b shows that animals fed a high-fat diet and treated with hGH (filled squares) or 20kDA hGH-V (filled circles) grew faster than animals treated with saline (open circles). Total change in body weight was significantly increased with hGH treatment (hGH versus saline; $p<0.0001$). Although the 20kDa hGH-V-treated animals grew faster than animals treated with saline, the effect was not statistically significant (20kDa versus saline; $p=0.3888$). Furthermore, treatment with hGH was associated with greater weight gain than treatment with 20kDa hGH-V (20kDA versus hGH; $p<0.0001$).

Daily Change in Body Weight

Figures 4a and 4b show the effects of hGH, 20kDa hGH-V and saline on animals fed either a normal diet (Figure 4a) or a high-fat diet (Figure 4b). Animals were treated with saline (open circles), hGH (filled squares) or 20kDa hGH-V (open squares). Figure 4a shows that treatment with hGH was associated with greater daily weight gain than treatment with 20kDa hGH-V or saline. Figure 4b shows that the effect of hGH was greater in animals fed a high-fat diet than in animals fed a normal diet (Figure 4a). Furthermore, there was an initial trend toward increased daily weight gain in animals treated with 20kDa hGH-V compared to animals treated with saline in animals fed either a normal diet (Figure 4a) or a high-fat diet (Figure 4b). However, by day 2, the difference was diminished.

Food and Water Intake

Food intake was initially increased in GH treated animals over the first 48 hours but subsequently returned to the intakes in saline- and 20kDa-treated animals by day 3 of treatment. There was no difference in water intake in any of the treatment groups.

Body Lengths

Nose anus length was significantly increased in animals fed the high fat diet ($p<0.05$) and were significantly increased in hGH animals compared to saline treated animals ($p<0.05$). The 20kDa placental hGH-V had little or no effect on length in animals fed a normal diet, but 20kDa hGH-V significantly increased body length in animals fed a high fat diet. Nose-to-tail lengths were significantly increased in hGH treated animals ($p<0.001$) but were not statistically different in the 20kDa treated animals compared to saline controls.

Fat Mass

Retroperitoneal fat was significantly increased in all treatment groups on the high fat diet. In Figure 5, the left-hand three columns represent results obtained in animals fed a normal diet. The right-hand three columns represent results in animals fed a high-fat diet. Open columns represent effects of saline, shaded columns represent effects of hGH and filled columns represent effects of 20kDa hGH-V. Animals fed a high-fat diet and treated with saline had a slightly increased retroperitoneal fat mass compared to animals fed a normal diet. Treatment with hGH or 20kDa hGH significantly decreased retroperitoneal fat mass compared to saline alone in animals fed a normal diet or a high-fat diet (Figure 5). The difference in fat mass between the hGH and 20kDa GH treatment groups was statistically significant for hGH versus saline ($p<0.001$) and for 20kDa GH-V versus saline ($p<0.005$), but was not statistically significant for 20kDa GH versus hGH ($p=0.4482$), although a trend was present. Overall, the effect of diet was significant ($p<0.05$) and for effect of treatment ($p<0.0001$).

Organ Weight

There was no effect of diet or hGH treatment on spleen, heart, kidney, brain, adrenal or testes weight. Liver weight (relative to body weight) was significantly decreased in high fat fed animal. However, liver size was significantly different between the 20kDa treated animals and those treated with either saline or hGH (20kDa GH versus saline; $p<0.005$; 20kDa GH versus hGH; $p<0.0001$).

Hematocrit

Figure 6 depicts a graph of effects of diet and hGH treatment on hematocrit (HTC; expressed as % of total blood sample volume). The left-hand three columns represent data from animals fed a normal diet. The right-hand three columns represent data from animals fed a high-fat diet. Open columns represent effects of saline, shaded columns represent effects of

hGH and filled columns represent effects of 20kDa hGH-V. There was a trend toward a decrease in HTC in animals fed a high-fat diet, but the effect was not statistically significant. However, treatment with hGH significantly reduced HTC in animals fed either normal diet or high-fat diet ($p<0.05$). There was a strong trend to decreased HTC in animals treated with 20kDa GH, but the effect was not statistically significant (saline versus 20kDa GH; $p=0.27$). Thus, there was a trend toward greater effect of hGH than for 20kDa hGH-V, but the effect was not statistically significant ($p=0.36$). Overall, although there was a slight trend to decreased HTC in animals fed a high-fat diet, the effect was not statistically significant ($p=0.53$). Further, although there was a trend to an overall effect of GH treatment, but the effect was not statistically significant ($p=0.14$).

Insulin

Figure 7 depicts a graph of fasting plasma insulin levels in animals fed either a normal diet (left-hand three columns) or a high-fat diet (right-hand three columns). Animals from each group were treated with saline (open columns), hGH (shaded columns) or 20kDa hGH-V (filled columns). There was a noticeable trend to an increase in fasting plasma insulin (expressed in $\mu g/l$) in saline treated animals on the high fat diet compared to animals fed a normal diet, but the effect was not quite statistically significant ($p=0.09$). In animals fed a low fat diet, hGH resulted in a slight decrease in plasma insulin (not statistically significant). Interestingly, in animals fed a high-fat diet, hGH was associated with a slight increase in plasma insulin ($p=0.76$). Thus, in animals treated with hGH, there was a dramatic increase in plasma insulin in animals fed a high-fat diet compared to animals fed a normal diet (more than a 2-fold increase). Quite surprisingly, however, no such effect was observed in animals treated with the 20kDa hGH-V. Thus, regardless of the diet, 20kDa hGH-V-treated animals had reduced plasma insulin levels compared to either saline-treated ($p<0.05$) or hGH-treated animals ($p<0.05$).

Glucose

There was no effect of treatment or diet on fasting plasma glucose concentrations although there was a trend towards lowering of plasma glucose concentrations in the 20kDa GH treated animals ($p<0.09$).

C-Peptide

Figure 8 depicts a graph of results of studies on fasting plasma C-peptide (expressed in μg/ml). The left hand three columns represent results obtained for animals fed a normal diet and the right-hand three columns represent results obtained for animals fed a high-fat diet. Open columns represent results obtained in saline-treated animals, shaded columns represent data obtained in hGH-treated animals, and filled columns represent data obtained in 20kDa hGH-V-treated animals.

Fasting plasma C-peptide concentrations were significantly increased in animals fed the high fat diet ($p<0.0005$, Figure 8). In animals fed a high-fat diet, treatment with hGH elevated C-peptide levels compared to saline-treated control animals, but the effect was not statistically significant ($p=0.29$). Further, in animals treated with hGH, plasma C-peptide increased to nearly 2.5 times that observed for animals fed a normal diet. This finding was similar to that observed for plasma insulin levels shown in Figure 7. Surprisingly, 20kDa hGH-V did not increase plasma C-peptide in either animals fed a normal diet or animals fed a high-fat diet. Rather, 20kDa hGH-V was associated with a modest decrease in plasma C-peptide in animals fed a normal diet. Quite surprisingly, 20kDa hGH-V-treatment abolished the increase in plasma C-peptide associated with conventional hGH treatment ($p<0.005$), and actually produced a statistically significant decrease in C-peptide compared to saline-treated animals fed a high fat diet ($p<0.05$). Overall, there was a statistically significant effect of treatment ($p<0.05$).

Free Fatty Acids (FFAs)

FFAs were significantly reduced in hGH- and saline-treated animals fed the high fat diet ($p<0.005$) as shown in Table 2. There was no change in FFA concentration in 20kDa hGH-V-treated animals fed either normal diet or high-fat diet (see Table 2). In fact, the difference in effects of hGH and 20kDa hGH-V was statistically significant ($p<0.05$).

Table 2. Free Fatty Acid Concentrations

Table 2. Free Fatty Acid Concentrations

Diet	FFA Concentration
Saline Normal Diet	1.19 ± 0.09mmol/l
hGH Normal Diet	1.06 ± 0.12mmol/l
20kDa Normal Diet	1.18 ± 0.13mmol/l
Saline High Fat Diet	0.78 ± 0.08mmol/l
hGH High Fat Diet	0.76 ± 0.08mmol/l
20kDa High Fat Diet	1.12 ± 0.05mmol/l

Overall, the effects of diet and treatment were statistically significant ($p<0.005$ and $p<0.005$, respectively). However, the effects of hGH and 20kDa hGH-V were not statistically different from results obtained in saline-treated animals ($p=0.11$ and $p=0.44$, respectively).

Triglycerides

Figure 9 depicts a graph of fasting plasma triglycerides (TG; expressed in mmol/l) in animals fed a normal diet (left-hand three columns) or a high-fat diet (right hand three columns). Open columns represent results obtained in saline-treated animals, shaded columns represent results obtained in hGH-treated animals and filled columns represent results obtained in 20kDa hGH-V-treated animals.

Plasma triglycerides were decreased in saline-treated animals fed a high-fat diet compared to animals fed a normal diet ($p<0.0001$). In animals fed a normal diet, hGH was associated with a slight increase in triglycerides, but 20kDa hGH-V-treatment significantly elevated triglyceride concentrations compared to hGH ($p<0.001$). Similarly, in animals fed a high-fat diet, hGH produced a slight increase in TG ($p=0.52$), and 20kDa hGH-V produced a greater increase than did hGH ($p<0.005$). There was an overall significant treatment effect ($p<0.005$).

Glycerol

There was a significant effect of the high-fat diet to decrease plasma glycerol levels ($p<0.005$), but no statistically significant effect of any treatment on plasma glycerol concentration was observed. Overall, the significance values for different comparisons were

as follows: treatment effect p=0.5; 20kDa hGH-V vs hGH p=0.66; 20kDa hGH-V vs saline p=0.49; hGH vs saline; p=0.26.

Total protein

High fat fed animals has significantly lower total protein concentrations than animals fed on a normal diet ($p<0.05$). Total protein concentrations were significantly reduced in the hGH- and 20kDa hGH-V- treated animals ($p<0.001$). Overall, the significance values for different comparisons are as follows: treatment effect; $p<0.005$; 20kDa hGH-V vs hGH; $p<0.05$; 20kDa hGH-V vs saline $p<0.005$; hGH vs saline; p=0.17.

Plasma Leptin

Fasting plasma leptin was significantly elevated in animals fed the high fat diet ($p<0.001$). There was no effect of hGH or 20kDa hGH-V on plasma leptin concentrations. Overall, the significance values for different comparisons are as follows: treatment effect; p=0.25; 20kDa hGH-V vs hGH; p=0.18; 20kDa vs saline; p=0.93; hGH vs saline; p=0.19.

Discussion

The inventors previously showed that the 20kDa hGH-V was as lipolytic as hGH and negated the increased plasma volume normally associated with hGH therapy (PCT/US2004/027187). The dwarf rat, although an ideal model for establishing efficacy of GH compounds in growth promotion, is not ideal for assessing changes related to the altered diabetogenic and lipid-related effects of hGH. The current trial was designed to confirm the preliminary data in outbread GH-normal rats on either a normal or high fat diet.

The growth promoting effects obtained for Wistar rats closely paralleled those observed in the GH-deficient dwarf rat. Although a comparatively weak growth promoter, the 20kDa placental GH was found to be as lipolytic as hGH. Further, 20kDa hGH-V did not cause a significant increase in plasma volume as was observed with hGH.

In a prior application, Serial No: 60/590,794 (Attorney Docket No: ERNZ 1016 US1) incorporated herein fully by reference, we found that otherwise normal but obese rats were suitable for studying diabetogenic effects of growth hormones. The results of the studies described herein indicate that one of the major side effects of growth hormone therapy using conventional growth hormones can be at least partially alleviated using a 20kDa hGH-V described herein.

Thus, effective treatment using 20kDa hGH-V can be carried out using doses of from about 0.1 to about 10.0mg/kg. Alternatively, a dose of about 1 mg/kg can be used. It can be appreciated that other doses outside this range can be tested without undue experimentation. Further, the duration of treatment with 20kDa hGH-V can be in the range of from about 1 treatment on one day to repeated treatments over a period 14 days. However, it can also be appreciated that one can treat subjects for longer periods of time without undue experimentation. Thus, the methods of this invention can produce desirable growth-promoting effects without producing undesirable side effects in subjects treated with growth hormone.

It can be appreciated that the above descriptions are for purposes of illustration only and are not intended to limit the scope of this invention. Rather, persons of ordinary skill can readily appreciate that modifications of the above methods and compositions can be readily used and prepared, and all such variations are considered within the scope of this invention. Further, all references cited herein are incorporated herein fully by reference.

References

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Incorporation by Reference

Each of the patents and articles cited herein are fully inforporated by reference. This application contains oligonucleotide and/or amino acid sequences. A Sequence Listing in computer readable form and on a diskette accompanies this application, and the Listing and the Sequences therein are incorporated herein fully by reference.

INDUSTRIAL APPLICABILITY

Embodiments of this invention are useful for treating mammals and in the manufacture of medicaments useful for treating mammals in need of growth hormone therapy for certain conditions. Other embodiments are useful for treating and in the manufacture of medicaments useful for treating mammals who have conditions or are at risk for conditions for developing a diabetogenic effect associated with growth hormone therapy.

What is claimed is:

1. A method of increasing levels of growth hormone in a mammal for prophylactic or therapeutic purposes, said mammal having or at risk for developing an adverse metabolic condition associated of growth hormone therapy, comprising administering to said mammal a pharmaceutically effective amount of 20kDa hGH-V or a polypeptide that is substantially identical to 20kDa hGH-V.

2. The method of claim 1, wherein the mammal suffers from adult-onset growth hormone deficiency.

3. The method of claim 1, wherein the mammal suffers from childhood-onset growth hormone deficiency.

4. The method of claim 1, wherein the mammal suffers from cystic fibrosis.

5. The method of claim 1, wherein the mammal suffers from osteoporosis.

6. The method of claim 1, wherein the mammal suffers from skeletal dysplasia.

7. The method of claim 1, wherein the mammal suffers from chronic kidney failure.

8. The method of claim 1, wherein the mammal suffers from depression.

9. The method of claim 1, wherein the mammal suffers from memory loss.

10. The method of claim 1, wherein the mammal suffers from a catabolic state.

11. The claim 1, wherein the mammal suffers from Turner's syndrome.

12. The method of any of claims 1 to 11, wherein the metabolic condition is selected from: diabetes mellitus, obesity, Prader-Willi syndrome, metabolic syndrome, insulin resistance, polycystic ovary syndrome, lipodystrophy or dyslipidemia.

13. A method of increasing levels of growth hormone in a mammal suffering from an adverse metabolic condition for prophylactic or therapeutic purposes, comprising administering to said mammal a pharmaceutically effective amount of 20kDa hGH-V or a polypeptide that is substantially identical to 20kDa hGH-V.

14. The method of claim 13, wherein the mammal suffers from hypertension, myocardial insufficiency, myocardial infarct or cardiac failure.

15. The method of claim 13, wherein the mammal suffers from one or more of diabetes mellitus, obesity, metabolic syndrome, insulin resistance, polycystic ovary syndrome, lipodystrophy or dyslipidemia.

16. The method of any of claims 1 to 15, wherein step of administering includes administering an expression vector capable of producing 20kDa hGH-V.

17. The method of any of claims 1 to 15, wherein said expression vector is in a host cell.

18. The method of claim 17, wherein said expression vector is in a cell of the patient.

19. A method for reducing diabetogenic effects associated with growth hormone therapy, comprising administering to a mammal in need of growth hormone therapy and having or at risk of developing a diabetogenic effect, a composition comprising 20kDa hHG-V.

20. The method of claim 19, wherein the step of administering includes administering to said mammal, a cell having a replicable vector therein capable of producing 20kDa hGH-V.

SEQ ID NO:1	ATG	GCT	GCA	GGC	TCC	CGG	ACG	TCC	CTG	CTC	CTG	GCT	TTT	GGC	CTG	CTC	TCG
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SEQ ID NO:3			GCA														
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		CCC															
		TCC															
		CCC															
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			TAC									CTA	GAA				
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			TAC				—	—	—	—	—	—	—	—	—	—	—
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		CCT				GTG	AAA	ACG	CAG			TCT					
		CCC				GAG	GAA	ACA	CAA			TCC					
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									TCA							CTC	
									TCG							TCT	

Figure 1

AGG	AGC	GTC	TTC	GCC	AAC	AGC	CTG	GTG	TAT	GGC	GCC	TCG	GAC	AGC	AAC	GTC							
	AGT								TAC			TCT											
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	AGT								TAC			TCT											
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Figure 1 (Cont.)

																				20
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	Pro	Ser	Asn	Arg	Val	Lys	Thr	Gln	Gln	Lys	Ser	Asn	Leu	Glu	Leu	Leu	Arg	Ile	Ser	Leu
					Glu	Glu														
					Val	Lys														
					Glu	Glu														
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	Leu	Leu	Ile	Gln	Ser	Trp	Leu	Glu	Pro	Val	Gln	Leu	Leu	Arg	Ser	Val	Phe	Ala	Asn	Ser
												Phe								
												Leu								
												Phe								

Figure 2

4/10

Leu Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr Arg/Asp/Arg/Asp His/Leu/His/Leu Leu Lys Asp Leu Glu Glu Gly (120)

Ile Gln Thr Leu Met Trp/Gly/Trp/Gly Arg Leu Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile Phe Asn/Lys/Asn/Lys (140)

Gln Ser/Thr/Ser/Thr Tyr Ser Lys Phe Asp Thr Lys/Asn/Lys/Asn Ser His Asn Asp Asp Ala Leu Leu Lys Asn Tyr (160)

Gly Leu Leu Tyr Cys Phe Arg Lys Asp Met Asp Lys Val Glu Thr Phe Leu Arg Ile Val (180)

Gln Cys Arg Ser Val Glu Gly Ser Cys Gly Phe (191)

Figure 2. (Cont.)

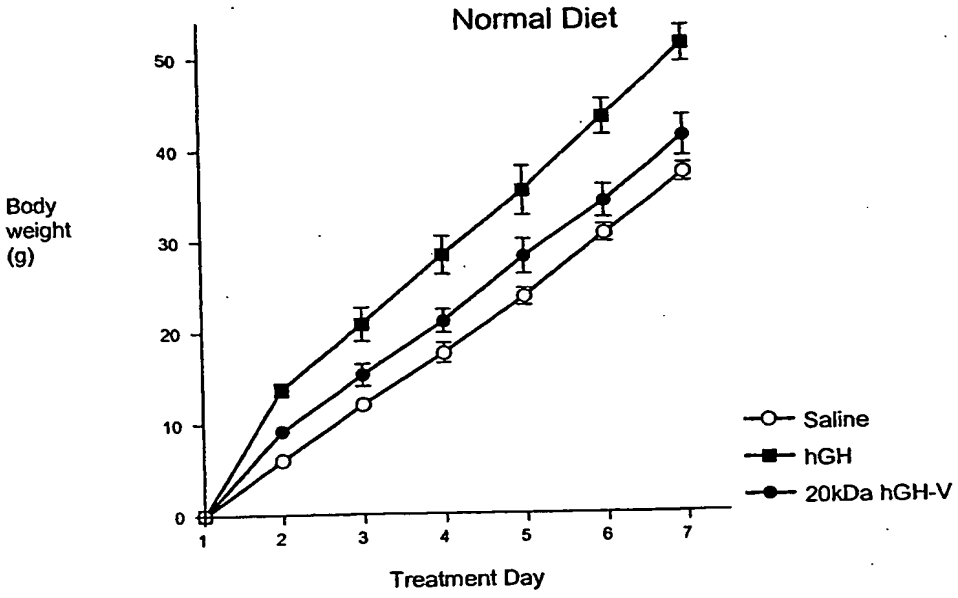


Figure 3a

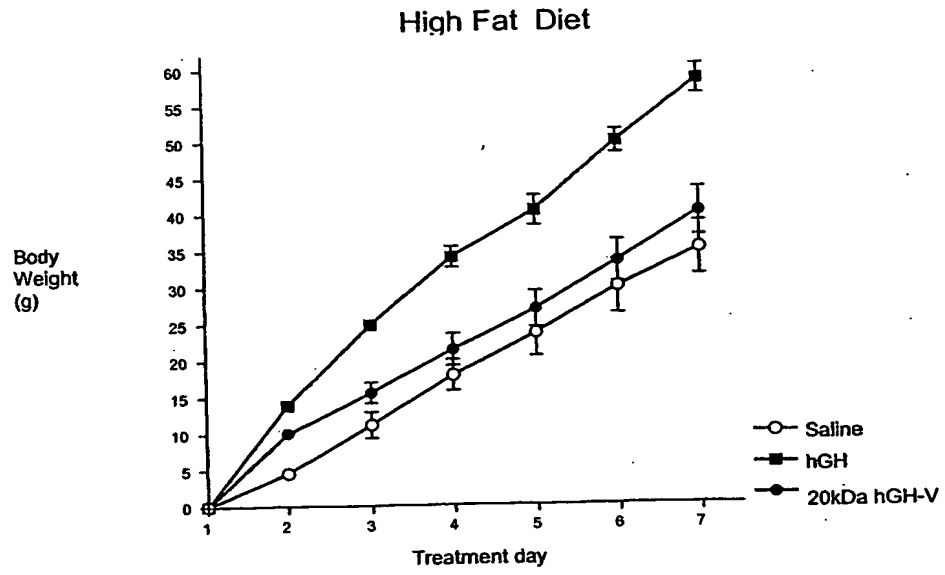
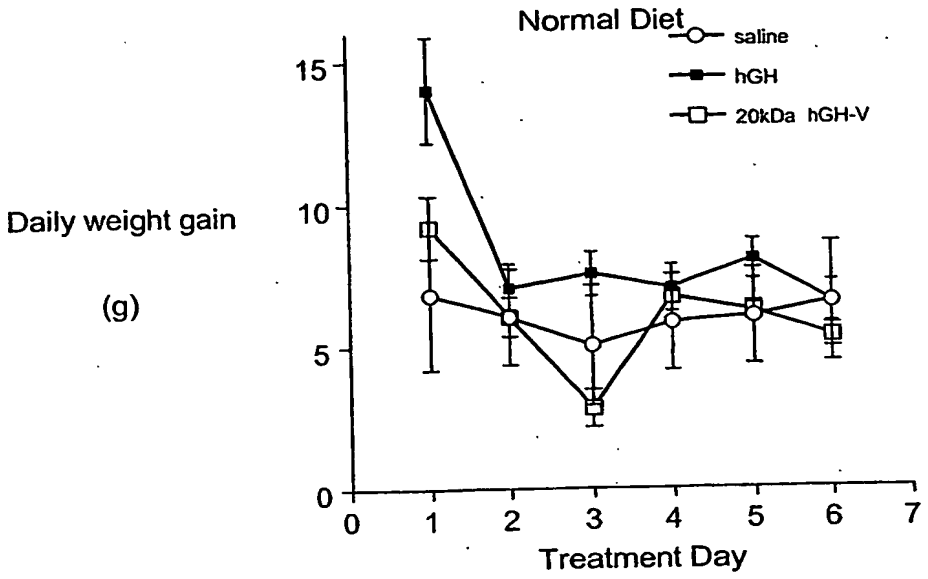


Figure 3b



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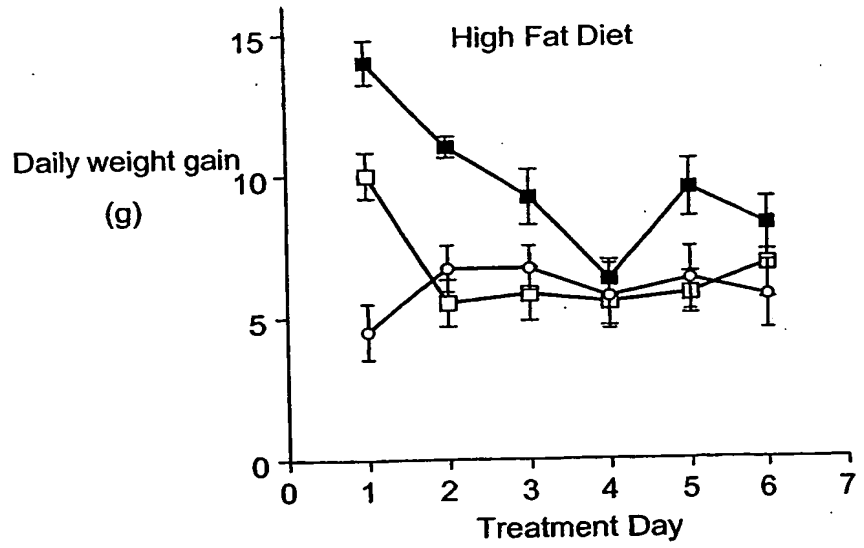

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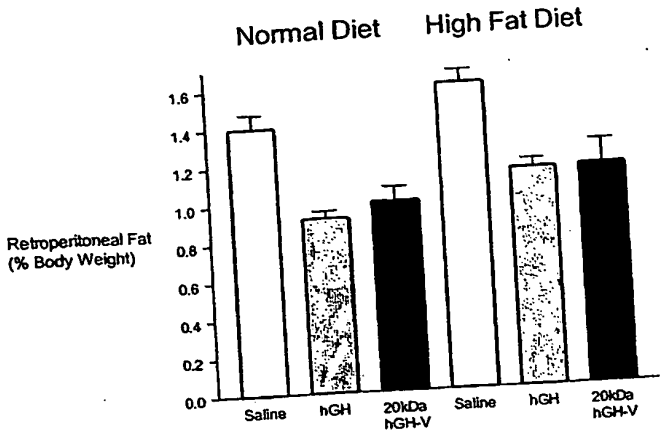

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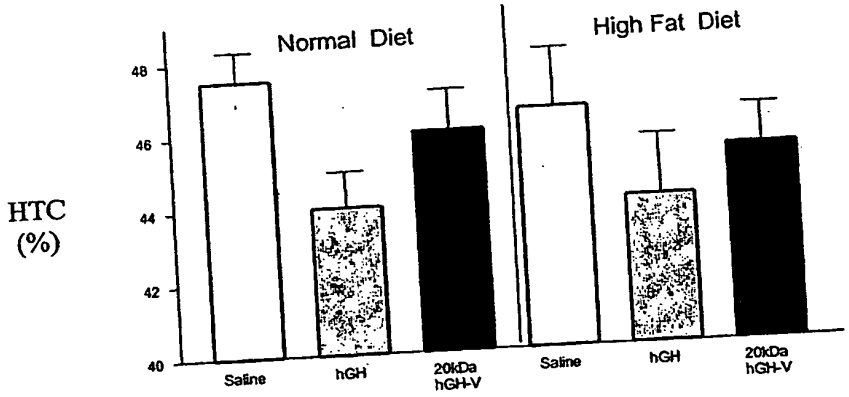
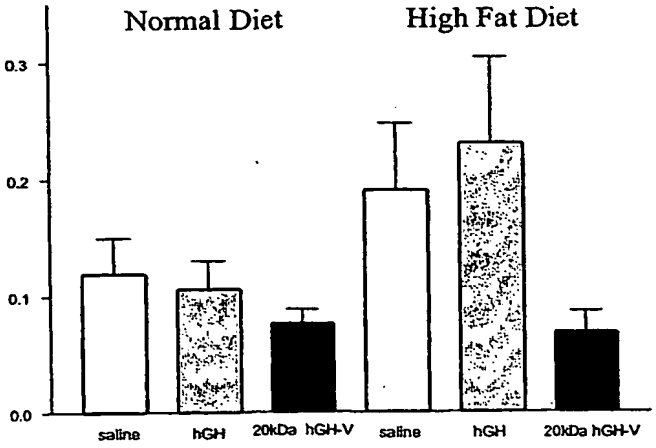


Figure 6

Plasma Insulin Concentration (μg/l)



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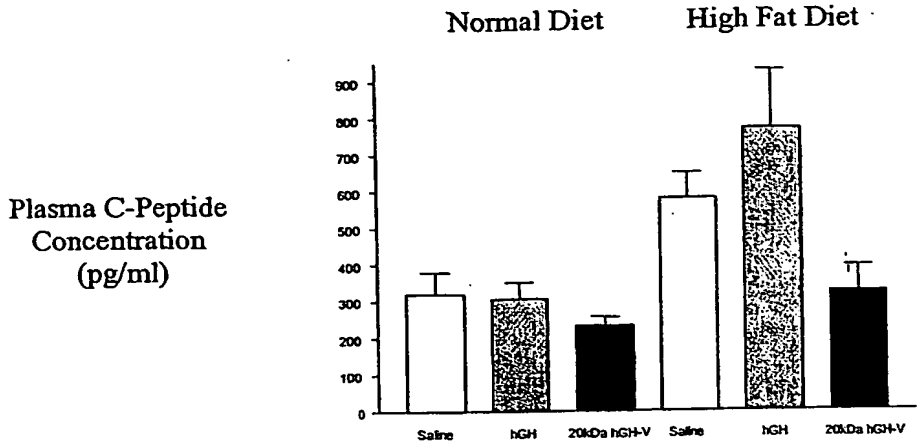
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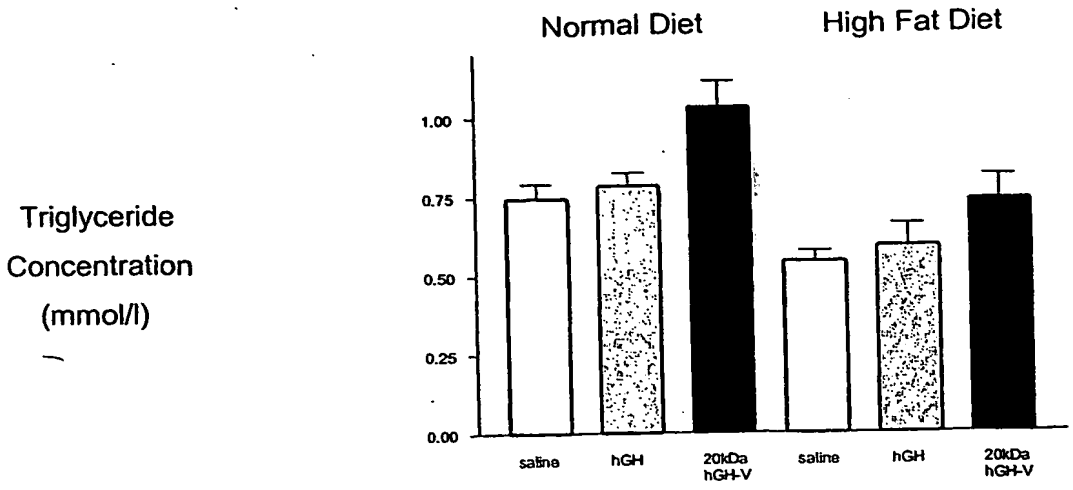
High Fat Diet
Plasma C-Peptide Concentration (pg/ml)
900
800
700
600
500
400
300
200
100
0
Saline
hGH
20kDa hGH-V
Saline
hGH
20kDa hGH-V

Figure 8


Normal Diet
High Fat Diet
Triglyceride Concentration (mmol/l)
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0.75
0.50
0.25
0.00
saline
hGH
20kDa hGH-V
saline
hGH
20kDa hGH-V

Figure 9

1016WO1.ST25.txt

SEQUENCE LISTING

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<120> Non Diabetogenic Therapy Using A 20kDa Placental Growth Hormone Variant

<130> ERNZ01016WO1

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Leu Leu Ile Gln Ser Trp Leu Glu Pro Val Gln Leu Leu Arg Ser Val
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His Leu Lys Asp Leu Glu Glu Gly Ile Gln Thr Leu Met Trp Arg Leu
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Gln Thr Ser Leu Cys Phe Ser Glu Ser Ile Pro Thr Pro Ser Asn Arg
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Glu Glu Thr Gln Gln Lys Ser Asn Leu Glu Leu Leu Arg Ile Ser Leu
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Leu Leu Ile Gln Ser Trp Leu Glu Pro Val Gln Phe Leu Arg Ser Val
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Phe Ala Asn Ser Leu Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr Asp
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Leu Leu Lys Asp Leu Glu Glu Gly Ile Gln Thr Leu Met Gly Arg Leu
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Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile Phe Lys Gln Thr Tyr Ser
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Lys Phe Asp Thr Asn Ser His Asn Asp Asp Ala Leu Leu Lys Asn Tyr
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Arg Val Lys Thr Gln Gln Lys Ser Asn Leu Glu Leu Leu Arg Ile Ser
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Val Phe Ala Asn Ser Leu Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr
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Arg His Leu Lys Asp Leu Glu Glu Gly Ile Gln Thr Leu Met Trp Arg
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